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MORPHOLOGY OF NORMAL PIGS' BLOOD[1]

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INTRODUCTION

The work reported in this paper was undertaken with the view of establishing normal data under conditions which exist in this section of the country as a basis for future studies and to corroborate the work of other investigators.

HISTORICAL REVIEW

Burnett[2] has compiled a table showing the number of erythrocytes, leucocytes, percentage of hemoglobin, specific gravity, and size of the red corpuscles in the blood of normal swine.

TABLE I.—*Summary of examinations of the blood of normal swine by different investigators (in Burnett[2])*

Red corpuscles per c. mm.	Leucocytes per c. mm.	Hemoglobin.	Specific gravity.	Size of red corpuscles.	Author.
		Per cent.		μ	
6, 960, 000	7, 840	....	....	5. 28–7. 9	Bethe.
7, 924, 000	19, 000	88	....	....	Giltner.
....	....	....	....	6	Gulliver.
5, 441, 000	....	....	....	....	Stöltzing.
8, 045, 000	....	....	....	....	Storch.
[a]4, 923, 000	11, 518	....	....	....	Do.
....	....	....	1, 060	6	Sussdorf.
8, 668, 200	....	....	....	....	Wendelstadt and Bleibtreu.

[a] Pigs 6–35 days old.

The number of examinations upon which the above averages are based is not known, except in the case of Giltner.[3] His original article is available, and his table shows that he made observations upon 24 pigs. From his description the pigs were of fair quality, and were of mixed breeding. Regarding size, they were quite mixed: Five were just weaned, one was a large animal, and the remainder varied in size.

Lewis, Shuler, and others,[4] in connection with their studies on hog cholera, have studied the blood of a few normal susceptible pigs. They

[1] Published, with the approval of the Director, as Paper No. 59 of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] Burnett, S. H. The Clinical Pathology of the Blood of Domesticated Animals. p. 48. Ithaca, N. Y., 1908.

[3] Giltner, Ward. The histology and physiology of normal pigs' blood. *In* Jour. Compar. Path. and Ther., v. 20, pt. 1, p. 18–23. 1907.

[4] Lewis, L. L., Shuler, W. P., McElroy, C. H., and Ritter, L. B. Hog cholera. Okla. Agr. Exp. Sta. Bul. 104, 30 p., 9 fig. 1914.

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find an average of 8,267,000 erythrocytes per cubic millimeter, 24,500 leucocytes, and 85 per cent of hemoglobin.

Dinwiddie,[1] in his bulletin dealing with a study of the blood of normal and cholera-infected pigs, reports upon 16 normal animals. These pigs were between the ages of 3 and 5 months. He found the average number of red corpuscles to be 6,334,000 and the leucocytes 11,800.

Differential counts of the leucocytes are reported by Drake,[2] Giltner,[3] and Dinwiddie.[4]

Drake, in his studies on trichinosis, made differential counts on 15 normal pigs. He recognizes only three classes of leucocytes. His results are as follows:

Lymphocytes	33 to 77 per cent;	average, 56.4 per cent.
Polynuclears	18 to 66 per cent;	average, 38.4 per cent.
Eosinophiles	1 to 12 per cent;	average, 5.13 per cent.

Giltner recognizes five classes of leucocytes and gives the following percentages based upon 24 examinations:

Lymphocytes	30.0 to 79.8 per cent;	average, 51.6 per cent.
Large mononuclears	.8 to 10.0 per cent;	average, 4.6 per cent.
Polymorphs	13.0 to 60.0 per cent;	average, 37.0 per cent.
Eosinophiles	1.2 to 11.0 per cent;	average, 5.2 per cent.
Mast cells	.2 to 5.6 per cent;	average, 1.3 per cent.

Dinwiddie gives the following percentages based upon 16 examinations:

Mononuclears	24 to 81 per cent;	average, 59.0 per cent.
Polymorphs	16 to 75 per cent;	average, 35.0 per cent.
Transitional	1 to 6 per cent;	average, 2.9 per cent.
Eosinophiles	1 to 10 per cent;	average, 4.0 per cent.
Basophiles	1 to 2 per cent;	average, 1.1 per cent.

METHODS OF STUDY

The 25 young pigs used in this work were obtained from three litters of pigs born from healthy sows and were farrowed on the university farm. They were healthy, strong pigs of mixed breeding. The pigs ranged in age from 2 to 42 days, and in weight from 2.5 to 18 pounds. The age, weight, sex, and condition are included in Table II.

The larger pigs were selected from animals which had been purchased upon the open market at the South St. Paul stock yards, and were to be used for serum production. The pigs came from various points throughout the Northwest, and when a load was received a number of the pigs were selected for this work. The pigs selected were of good quality. They were first weighed and tagged, then given a few days' rest in a small inside pen. During this time they received feed and water three

[1] Dinwiddie, R. R. Studies on the hematology of normal and cholera-infected hogs. Ark. Agr. Exp. Sta. Bul. 120, p. 21-41, 8 fig. 1914.

[2] Drake, A. K. Trichinosis. *In* Jour. Med. Research, v. 8 (n. s. v. 3), no. 1, p. 255-267, 1 pl. 1902.

[3] Giltner, Ward. Op. cit.

[4] Dinwiddie, R. R. Op. cit.

times daily. If the pigs showed any sickness, as evidenced by loss of appetite or increase in body temperature, they were not used. All of the pigs listed in Table III were afterwards used in producing hog-cholera virus.

The young pigs were taken from the pen, in which they were confined with their mother, to the operating room, and the various tests made or samples collected; following this, the young pigs were returned to the mother. The samples of blood were taken from the small veins in the ear, after applying alcohol and then shaving. The puncture was made with a sharp scalpel.

The samples from the larger pigs were obtained in a similar manner, except that these pigs were confined in a special hog crate. The samples were obtained as nearly as possible at the same hour each day, 10 a. m.

The puncture produced a good flow of cutaneous blood, and the first few drops were wiped away. It was usually necessary to make several punctures before completing the examination.

The usual methods of study were employed. The percentage of hemoglobin was obtained by means of the Sahli hemometer. The blood for estimating the number of erythrocytes and leucocytes was drawn in the same pipette, and Toisson's diluting fluid used as the diluting mixture. Two pipettes giving a dilution of 1 to 100 were used in each case, and both erythrocytes and leucocytes were counted on the same field. A count was made from each pipette, and, if the amount of variation was 100,000 erythrocytes or less, the results were considered satisfactory and were recorded. The counts were made on the Thoma-Zeiss hematocytometer counting chamber with the Zappert-Ewing ruling. The leucocytes were sometimes estimated by the acetic-acid method, with a pipette of 1 to 10 dilution. The specific gravity was determined by the Hammerschlag method. The time of coagulation was determined by means of the Biffi-Brooks coagulometer.

In making the differential counts of the leucocytes Wright's stain was employed as the staining agent, and 200 to 300 of the corpuscles counted on at least two spreads of blood, so that never less than 500 leucocytes were counted.

RESULTS OF EXPERIMENTATION

Twenty-five examinations were made of the blood of normal pigs between the ages of 2 and 42 days. The results of these examinations are shown in Table II. The average number of erythrocytes was 3,855,000 per cubic millimeter, and 13,500 leucocytes per cubic millimeter. The average clotting time was 64 seconds, specific gravity 1.024, and the average hemoglobin percentage 56.8. Differential count of leucocytes showed the following: Lymphocytes, 63.25 per cent; polymorphs, 32.14 per cent; mononuclears, 2.63 per cent; eosinophiles, 1.28 per cent; mast cells, 0.24 per cent.

TABLE II.—*Details of blood studies on 25 young pigs*

Description of animal.					Blood.					Differential count.				
No.	Age.	Sex.	Weight.	Condition.	Clotting time.	Hemoglobin.	Specific gravity.	Number of erythrocytes.	Number of leucocytes.	Lymphocytes.	Polymorphs.	Mononuclears.	Eosinophiles.	Mast cells.
	Days.		*Lbs.*		*Secs.*	*P. ct.*				*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
1	2	Male	2.5	Good	....	55	....	2,864,000	8,000	48.38	47.44	3.03	0.94	0.18
2	5	...do	6.0	Very good.	70	57	....	3,598,000	15,000	63.46	31.45	4.52	.56	.0
3	5	...do	5.0	Good	50	58	....	2,560,000	22,000	51.18	44.29	4.13	.39	.39
4 [a]	5	...do	....	...do	....	62	....	....	....	49.00	43.50	5.22	1.08	.09
5 [a]	5	...do	....	...do	....	70	....	2,240,000	22,000	79.50	18.00	2.70	1.0	.8
6	7	Female	6.0	...do	....	50	....	3,464,000	10,000	72.40	25.17	1.97	.01	.0
7 [a]	7	...do	....	...do	....	37	....	2,308,000	5,000	63.50	30.50	3.00	.42	1.6
8	8	...do	5.0	...do	60	55	....	3,068,000	11,000	58.95	38.21	2.18	.65	.0
9	11	...do	6.0	...do	60	50	....	4,300,000	13,000	58.76	40.07	1.15	.0	.0
10	15	Male	7.0	...do	60	45	....	3,315,000	....	75.73	22.81	1.27	.18	.0
11	19	...do	8.0	...do	80	40	1.035	3,378,000	10,000	84.70	14.57	0.36	.36	.01
12	22	...do	8.0	...do	....	38	1.013	2,528,000	11,000	85.02	13.67	0.92	.37	.0
13 [a]	28	...do	12.0	...do	65	85	....	4,376,000	10,000	76.00	20.40	1.80	1.10	.33
14 [a]	28	Female	15.0	...do	60	67	....	4,688,000	18,000	77.00	15.00	3.90	2.70	.0
15 [a]	28	Male	18.0	...do	....	90	....	5,220,000	20,000	80.20	6.30	2.80	8.50	.1
16	28	...do	8.0	...do	....	47	....	4,970,000	....	60.00	37.30	2.70	.0	.0
17	32	...do	10.0	...do	60	60	....	5,994,000	6,000	51.00	44.00	0.025	.018	.002
18	33	Female	10.0	...do	40	50	....	2,420,000	11,000	58.13	33.70	3.50	.46	.0
19	33	Male	10.0	...do	50	65	....	4,784,000	10,000	66.42	27.28	2.87	1.07	.52
20 [a]	35	Female	....	...do	....	46	....	2,680,000	10,000	56.00	40.00	2.20	1.40	.70
21	35	...do	10.0	...do	60	45	....	4,602,000	13,000	61.40	34.88	1.86	1.40	.46
22	37	...do	14.0	Very good.	60	65	....	5,032,000	12,000	52.93	43.15	2.93	.81	.16
23	40	...do	13.0	Good	90	60	....	4,622,000	15,000	60.60	35.40	3.00	1.00	.0
24	40	Male	10.0	...do	60	65	....	4,380,000	20,000	33.79	63.39	3.63	.17	.0
25	42	...do	10.0	...do	100	65	....	5,136,000	26,000	57.70	33.93	4.09	3.44	.82
Average	....	....	....	....	64.0	56.8	1.024	3,855,000	13,500	63.25	32.1365	2.63	1.2872	.246

[a] Examinations made by Dr. L. E. Willey.

Twenty-five examinations were made upon the blood of pigs weighing in the neighborhood of 100 pounds. The results of this examination are shown in Table III. The average number of erythrocytes per cubic millimeter was 6,215,000, and the average number of leucocytes per cubic millimeter was 18,000. The average clotting time was 57.60 seconds, specific gravity 1.062, and the average hemoblogin percentage was 79.4. The differential count of leucocytes showed the following: Lymphocytes, 55.21 per cent; polymorphs, 39.79 per cent; mononuclears, 0.79 per cent; eosinophiles, 3.42 per cent; mast cells, 0.79 per cent.

Table III.—*Details of blood studies on 25 older pigs*

Description of animal.					Blood.					Differential count.				
No.	Weight.	Sex and color.	Condition.	Temperature.	Clotting time.	Hemoglobin.	Specific gravity.	Number of erythrocytes.	Number of leucocytes.	Lymphocytes.	Polymorphs.	Mononuclears.	Eosinophiles.	Mast cells.
	Lbs.				*Sec.*	*P. ct.*				*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
6618	77	White and black female.	Good..	.....	45	70	1.046	6,536,000	15,000	62.15	34.72	0.86	2.08	0.17
6627	99	Red and black female.	...do..	.....	45	55	1.064	5,798,000	14,000	48.78	47.96	1.46	1.62	.16
6692	88	White female.	...do..	.....	70	48	1.046	5,656,000	24,000	47.89	50.42	.16	.84	.67
6714	70	.....do......	...do..	.....	50	75	1.027	6,344,000	17,000	69.85	28.49	.18	.55	.90
6772	92	.....do......	...do..	.....	50	74	1.053	6,704,000	17,000	66.90	28.93	.36	3.43	.47
6765	97	Red female..	...do..	.....	60	72	1.048	6,012,000	15,000	59.68	37.20	1.21	1.90	.00
5991	80	Brown and black male	...do..	.....	60	105	1.060	7,268,000	15,000	55.88	42.40	123	2.47	.00
6032	76	.....do......	...do..	.....	60	90	1.065	7,080,000	16,000	33.50	64.39	1.32	.75	.00
6224	88	Black female	...do..	.....	100	72	1.050	5,658,000	20,000	38.81	56.37	1.09	2.21	1.47
6225	86	.....do......	...do..	.....	90	80	1.063	6,872,000	19,000	51.68	38.94	2.83	6.19	.35
6981	74	Black and white female.	...do..	102.8	65	70	1.070	5,640,000	14,000	50.65	46.75	1.30	.52	.78
7073	101	White male.	...do..	99.3	70	70	1.063	5,156,000	33,000	42.40	51.42	.16	5.52	.47
7015	101	Female......	...do..	102.4	70	78	1.070	6,831,800	14,000	60.90	25.73	.00	10.00	3.34
7071	108	Red female..	...do..	102.4	70	65	1.069	6,196,000	17,000	50.97	46.09	.42	2.37	.14
7160	85	White male.	...do..	101.6	55	70	1.064	5,272,000	19,000	42.25	55.36	1.21	.00	.17
7162	88	White female.	...do..	103.2	40	70	1.070	5,456,000	19,000	53.09	43.43	.58	2.31	.58
7216	111	Black male..	...do..	103.6	70	70	1.066	5,520,000	18,000	56.92	35.86	.19	5.88	1.14
7217	102	Black female	...do..	102.8	35	70	1.071	6,380,000	15,000	62.83	28.32	.36	5.13	3.37
7273	105	Black male..	...do..	102.4	55	70	1.067	5,960,000	15,000	70.62	24.48	.19	4.33	.37
7271	105	Black female	...do..	103.6	35	70	1.070	6,104,200	18,000	68.43	25.42	.34	4.26	1.53
7272	130	.....do......	...do..	103.5	20	100	1.066	5,624,000	28,000	55.98	38.61	.38	4.82	.19
6308	110	White female	...do..	102.2	60	115	1.071	7,568,000	17,000	58.88	40.18	.37	.52	.00
7276	110	.....do......	...do..	102.8	75	112	1.069	6,217,000	20,000	.....	.....	.....	.....	.....
7278	110	Black and white male	...do..	101.8	60	102	1.066	5,976,000	20,000	57.05	26.61	1.40	13.11	2.09
7279	120	Red female..	...do..	103.3	30	112	1.070	7,580,000	19,000	59.17	37.04	1.54	1.37	.85
Average..	.....	....	....	.....	57.60	79.40	1.06176	6,215,160	18,320	52.21	39.79	.79	3.42	.79

In making the differential counts we have used a classification of the leucocytes similar to the one proposed by Giltner.[1] Drake[2] recognizes only three varieties of leucocytes, but we agree with Giltner in that five varieties can be easily distinguished in a well-made and well-stained spread. Dinwiddie[3] also recognizes five classes. He classifies all the mononuclear elements under one head, and gives the transitional forms a special classification.

The following is a somewhat detailed description of the various classes observed in our studies.

Lymphocytes.—Burnett[4] states that the cells falling ir this group have practically the same appearance in the majority of domesticated animals. With Wright's stain the cell body has a greenish blue tint, while the nucleus has a dark-violet tint. Giltner[1] describes two kinds

[1] Giltner, Ward. Op. cit.
[2] Drake, A. K. Op. cit.
[3] Dinwiddie, R. R. Op. cit.
[4] Burnett, S. H. Op. cit., p. 35.

of lymphocytes in the blood of normal pigs, the first variety slightly larger than the red cells, generally spherical in shape, from 7.4 to 10 μ in diameter, and averaging about 8.5 μ. They have a nucleus which occupies nearly the whole cell, showing in most cases a crescent-shaped darker or lighter blue portion of the cell body at the periphery. The second variety includes much larger cells, 11 to 14 μ in diameter. They are fewer in number, have round nuclei, and occupy relatively less of the cell space.

Giltner's classification, therefore, is the same as the classification of large and small lymphocytes often referred to in the literature when discussing this particular class of cells in other animals.

In our studies we have been able to recognize three classes of lymphocytes. The cells of the first class are few in number and about one-half the size of a red cell. The nucleus is round, deeply stained, and occupies almost the entire cell space. These cells were observed in the blood of both young and older animals. The cells of the second class, the most numerous by far, are a little larger. They are two to three times as large as a red cell, the nucleus is stained dark, and a small amount of bluish green cytoplasm can be recognized. The cells of the third class of lymphocytes are still larger; they have a slightly larger nucleus and a larger amount of visible protoplasm. It is sometimes difficult to distinguish between these cells and the smaller of the so-called large mononuclears.

No attempt was made to work out the various percentages of these three classes of lymphocytes; they were, therefore, all classed as lymphocytes. It would be extremely difficult to work out the percentages of these three classes, and the results would be subject to much error, owing to the fact that many border-line cells present themselves, and it would be difficult to know into just what class to place some of them.

LARGE MONONUCLEARS.—Burnett states these cells are usually about twice the diameter of the average red corpuscles. The nucleus usually occupies only about one-half of the cell and is situated on one side of the center. Its shape is oval or curved. Both nucleus and cell body are finely reticular and stain less deeply than do those of the lymphocytes. The cell body is faintly basophile. The cells have much the same appearance in the several species of domestic animals.

Giltner [1] states that in the pig the more typical large mononuclear leucocytes are similar in size to the large lymphocytes and have a medium-sized nucleus, light blue and bean-shaped, and show a large portion of the cell body stained a different shade of blue.

The cells which we have classified as large mononuclear leucocytes vary in size from a size equal to, or a little larger than, the largest lymphocytes up to very large cells, the largest of which are 5 times as long and 5

[1] Giltner, Ward. Op. cit.

times as wide as an average red corpuscle. In recognizing these cells two things are important: First, the great size of some of the cells, and second, the lighter stained nucleus than the lymphocytes and the larger amount of visible cytoplasm. This cytoplasm in a well-stained spread is light bluish green in color, with a few fine darker granules throughout.

We have found that it is sometimes difficult to determine whether to place some of these mononuclear cells into the class of lymphocytes or large mononuclears, as there are quite a number of border-line cells.

POLYMORPHONUCLEARS.—The nucleus in this variety is several-lobed; the lobes are nearly always connected, sometimes with threadlike connections, but more often the connecting portions are as wide as the lobes, so that the nucleus assumes the form of a deeply stained spiral coil. It may be roughly S-shaped or Z-shaped. In well-stained spreads the cell body contains many fine granules so small that they appear as brick-red points. The entire cell is about the size of the largest lymphocytes. Sometimes the lobes of the nucleus are not connected and stand out in such a way that they can be easily counted, and average about six to seven in number.

EOSINOPHILES.—Giltner states that the eosinophiles are comparable to the polymorphs in size, or are slightly larger, and have a bilobed nucleus, the two parts of which are connected by a thick band and take the basic stain. The cell body is granular, but the granules are not nearly so large and distinct as those found in the eosinophile of the horse, but are more numerous (estimated at 100, more or less) and have a strong affinity for the eosin stain.

In our work we have found that the eosinophiles are usually slightly larger than the polymorphs. The nucleus is darkly stained, but is lighter than the nucleus of the polymorphs. The lobes are wide, and there may be one to four lobes in each nucleus. The lobes are usually connected by broad bands, but we sometimes find a nucleus containing two large lobes which are not connected. No attempt was made to count the number of granules.

MAST CELLS.—Giltner [1] states the mast cells in the blood of the pig are about the same size as the eosinophiles and have a similar-shaped nucleus, but that the cell body possesses granules of a smaller size, more distinct in outline, and of a purple color. The granules lie both in the cytoplasm surrounding the nucleus and in a position superposed to the nucleus.

In the blood of some pigs we have found mast cells about the size of eosinophiles, which closely resemble a large lymphocyte except that the space in the cell body not occupied by the nucleus is filled with dark-blue granules. Granules are also quite numerous over the nucleus in many of these cells, but the number of granules placed in this location

[1] Giltner, Ward. Op. cit.

varies widely. These granules are not as large as the red granules found in the eosinophiles.

Gruner[1] speaks of this kind of a mast cell as a true mast cell. Such cells have a narrow cytoplasm. The granules vary in size. He states that these cells are lymphocytes which have undergone mucoid degeneration.

In the blood of some pigs the mast cells are quite large, the nucleus usually bilobed, a large amount of cell space is visible, and the granules are slightly larger but not as numerous as in the cells first described.

TRANSITIONALS.—Dinwiddie[2] recognizes a classification of transitional leucocytes. Occasionally true and distinct transitional forms of leucocytes are met with in the blood of the pig, but we have followed the custom of most workers and have classified them in one group of the related type. True transitionals would very likely represent about 1 to 2 per cent of the total leucocytes, but there would be many border-line cases, and it would be difficult to know just where to place them. Individual workers would very likely differ widely in classifying them, and we have found it much easier to place them in the recognized group which they most closely resembled. Therefore, when these cells were encountered, they were either added to the large mononuclears, lymphocytes, or polymorphs.

BLOOD PLATELETS.—No attempt was made to estimate the number of blood platelets per cubic millimeter. Blood plates occurring singly and in clumps are often encountered in the dry spread. When Wright's stain is used, the blood plates take a bluish tint and show many dark granules. The blood platelets which are encountered singly vary in size and shape. They may be round, oval, or irregular; some are smaller, and others are larger than an average-sized red corpuscle. When in clumps, the individual size, shape, etc., can not be determined.

ERYTHROCYTES.—In a well-stained spread the erythrocytes are red in color and are circular. Willey, working in this laboratory, found nucleated red corpuscles in the blood of a 4-weeks-old pig.

EFFECTS OF AGE.—Observations made by different workers relatively to the effects of age upon the number of red blood cells are conflicting. Storch[3] reports the number in adult swine to be 8,045,000 and in young pigs (6 to 35 days old) 4,923,000.

Giltner's[4] table shows a higher red-cell count in young pigs (2.5 months) than in the older animals, averaging 8,363,333 erythrocytes for the young and 7,322,500 for the older. The leucocytic count was about the same in the two groups.

Our results are more in accord with those of Storch. The red corpuscles showed an average of 3,853,071 cells per cubic millimeter in the

[1] Gruner, O. C. Biology of the Blood-Cells . . . p. 259. London, 1913.
[2] Dinwiddie, R. R. Op. cit.
[3] Burnett, S. H. Op. cit., p. 48. Cites paper by Storch.
[4] Giltner, Ward. Op. cit.

25 young pigs and 6,176,272 in the older hogs. The leucocytic count was also lower in the young animals, averaging 12,328 in the young and 18,533 in the older animals.

Burnett[1] states that the lymphocytes in young animals are generally present in greater numbers than in adults. He states that in puppies 3 to 20 days old they were 20.8 to 30.7 per cent, while for adults they average 19.4 per cent. We have found a similar condition in the pig. In young pigs the lymphocytes average 62.25 per cent and in adults 55.21 per cent. The large mononuclears are also present in greater numbers in the young animals, 2.63 per cent being found in young animals and 0.75 per cent in older animals. The polymorphs, eosinophiles, and mast cells are present in greater numbers in adults. These cells show a percentage of the polymorphs of 39.79 in older animals and 32.13 in young animals; of the eosinophiles, 3.42 in the old animals and 1.28 in the young; of the mast cells, 0.79 in old animals and 0.24 in the young.

Giltner[2] found a higher percentage of hemoglobin in the older animals, 88 in the older and 85 in the younger.

Our results also show a higher percentage of hemoglobin in the older animals. The older animals averaged 79 per cent, as compared with 66 per cent in the younger animals.

We also found a higher specific gravity in the case of the older pigs, and the clotting time was about 6 seconds shorter in these older animals.

Differences of sex.—Burnett[3] states that the number of red corpuscles and the amount of hemoglobin seem to be higher in male than female domestic animals.

Giltner's results show about the same number of red corpuscles in the blood for females and males. They average 7,945,000 for the males and 7,895,500 for the females. He found the leucocytes to be a little higher in the male. These cells average 18,150 in the male and 16,415 in the female. Giltner found the percentage of hemoglobin to be about equal in the two sexes, 87 in the male and 88 in the female.

Our results show the number of erythrocytes to be about equal in both groups (young and old). In young pigs the average number of red corpuscles was 3,953,071 in the males, and 3,718,400 in the females. In the older pigs the average number of red corpuscles was 6,068,250 in the males, and 6,284,294 in the females. We found a higher leucocytic count in the males than females. The young males showed an average white-cell count of 12,857, as compared with 11,800 in the females. The older males showed an average white-cell count of 19,125, as compared with 17,941 in the females.

We found a higher percentage of hemoglobin in the male animals of both groups. In the case of the young animals the males showed 60 per cent, as compared with 52.5 per cent in the female. The older males

[1] Burnett, S. H. Op. cit. [2] Giltner, Ward. Op. cit. [3] Burnett, S. H. Op. cit., p. 58.

showed an average of 81.5 per cent as compared with 78 per cent in the older females. The average differential counts for the two sexes showed no important differences (Table IV).

TABLE IV.—*Variation in the differential count in male and female pigs*

Age.	Male.					Female.				
	Lymphocytes.	Polymorphs.	Large mononuclears.	Eosinophiles.	Mast cells.	Lymphocytes.	Polymorphs.	Large mononuclears.	Eosinophiles.	Mast cells.
Young..	64. 14	31. 22	2. 67	1. 28	0. 216	61. 96	33. 61	2. 57	1. 30	0. 29
Old......	53. 81	41. 12	. 73	4. 07	. 64	56. 05	39. 13	. 83	3. 10	. 87

SUMMARY

(1) The number of erythrocytes in the blood of the pig varies under different conditions. It is lower in young animals than in old.

(2) The number of erythrocytes also varies according to the condition of the animal. A well-nourished pig in good condition will show a higher count than a pig in poor condition and of the same age.

(3) The number of erythrocytes was about equal in the blood of male and female animals.

(4) The leucocyte count was lower in young animals, but individuals of the same class may show considerable variation.

(5) The number of leucocytes seems to be higher in male than in female animals.

(6) The percentage of hemoglobin was higher in older animals.

(7) The percentage of hemoglobin was higher in male than female animals.

(8) The specific gravity of the blood was higher in older animals.

(9) The clotting time was less in younger animals.

(10) Five classes of leucocytes can be recognized in the blood of the pig: Lymphocytes, large mononuclears, polymorphonuclears, eosinophiles, and mast cells.

(11) Results of the differential counts by various workers are fairly uniform.

(12) The percentage of lymphocytes and large mononuclears is higher in young animals.

(13) Older animals show a higher percentage of polymorphonuclears, eosinophiles, and mast cells than young animals.

(14) Differential counts in male and female animals are about the same.

FIXATION OF AMMONIA IN SOILS[1]

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INTRODUCTION

It is well known that the capacity of a soil to serve as a reservoir for plant food depends in a large measure upon its power to retain water-soluble substances, such as potash, phosphoric acid, and ammonia, against the leaching action of rains.

There are undoubtedly many forces which operate in the retention of soluble salts in soils, and it is quite likely that these forces are very different in different soils. The extremely complicated nature of the phenomena has led to many apparently conflicting observations; and it would seem that an entirely satisfactory explanation of the fixation processes can not be secured until more refined methods have been developed which will make possible a more exact knowledge of the physical and chemical forces involved.

In studying the nitrifying power of semiarid soils at different depths, it was observed that, when ammonium sulphate was added to soils drawn from considerable depths, little or no increase in nitrates resulted during the first two weeks' incubation; but, notwithstanding the lack of nitrification, the ammonia added could not be recovered from the soil by any of the ordinary methods for determining ammonia in soils. In the early work only small quantities of ammonium sulphate were added, and it was thought possible that the ammonia might have been assimilated by microorganisms. Larger quantities of ammonium sulphate were then added. Little or no increase in nitrates was secured, but only a small percentage of the ammonia added could be recovered from the soils drawn from a considerable depth. The results obtained with the surface soil were quite different, about 95 per cent of the nitrogen added being recovered as ammonia or nitrates. This observation seemed to indicate a strong ammonia-fixing power for the deep soil layers, a condition which, if true, would lead to complications in studying the ammonifying and nitrifying power of the soils at different depths.

The work reported in this paper is limited to a study of the fixation of ammonia by soils, and is an outgrowth of the observations stated above.

[1] The work discussed in this paper was carried out in cooperation with the University of California Citrus Experiment Station and Graduate School of Tropical Agriculture at Riverside. The writer wishes to express his indebtedness to Director H. J. Webber and members of his staff for many courtesies and facilities extended during the course of the work.

METHODS OF EXPERIMENTATION

The soil samples employed in the investigations reported in this paper were secured by means of soil-sampling tubes or, where large quantities of soil were desired, by digging a hole to the desired depth and removing a uniform block of soil from the side. All the soils studied were passed through a coarse sieve, after which they were thoroughly mixed. One-hundred-gm. portions of dry soil were then weighed into 1-quart

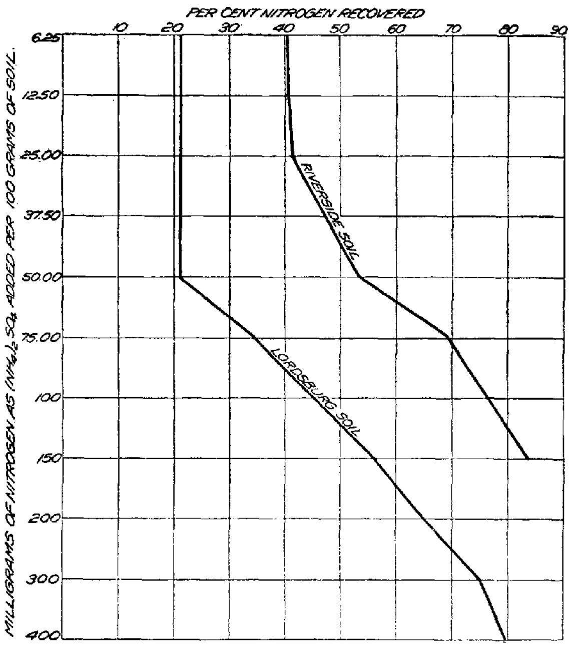

FIG. 1.—Diagram showing the percentage of ammonia recovered from soils when increasing amounts of ammonium sulphate were added.

Mason jars. The desired amount of ammonium salts or other salts was added in standard solutions in such quantities that the solution added was sufficient to saturate the soil thoroughly.

Unless otherwise stated in the text, the ammonium solution was allowed to remain in contact with the soil for 30 minutes. The soil was then extracted by a 10 per cent hydrochloric-acid solution and an aliquot portion of the extract, rendered alkaline by adding sodium-

hydroxid solution, was distilled. The distillate was titrated with alizarin.

The acid-extraction method was used in preference to direct distillation with magnesium oxid, because duplicate determinations by acid extraction gave a much closer agreement than could be secured by distilling the soil with magnesium. Ten per cent of acid was used, as it was found that this amount of acid was necessary to extract as much ammonia as would be given off by distilling with magnesium oxid.

The results stated in the following tables are averages of a number of determinations. In no case is a result for a single analysis given, and in some instances the figures as given are averages of six or more determinations.

COMPARISON OF METHODS FOR RECOVERY OF AMMONIA FROM SOILS

Fifty mgm. of nitrogen in the form of ammonium sulphate, chlorid, or nitrate were added to 100-gm. portions of soil from Riverside, Cal. After standing for 30 minutes, the soils were treated as shown in Tables I and II. When the soil was extracted with water only, but little more than one-fourth of the nitrogen added was recovered. When extracted with increasing strengths of hydrochloric acid, the percentage of nitrogen recovered increased with the strength of the acid; but even when the acid solution was increased to 10 per cent, only a little more than half of the nitrogen added was recovered.

TABLE I.—*Recovery of ammonia from soil by extracting with water and increasing the amount of hydrochloric acid*

Soil extracted with—	Nitrogen added per 100 gm. of soil.	Nitrogen recovered from ammonium sulphate.		Nitrogen recovered from ammonium chlorid.		Nitrogen recovered from ammonium nitrate.	
	Mgm.	*Mgm.*	*Per cent.*	*Mgm.*	*Per cent.*	*Mgm.*	*Per cent.*
Water only	50.00	12.88	25.76	13.28	26.56	13.00	26.00
1 per cent acid	50.00	22.12	44.24	22.33	44.66	22.30	44.60
2½ per cent acid	50.00	22.68	45.36	23.30	46.60	23.20	46.40
5 per cent acid	50.00	24.36	48.72	24.74	49.48	24.50	49.00
10 per cent acid	50.00	26.04	52.08	25.76	51.52	25.76	51.52

The acid-extraction method and the magnesium-oxid method for determining ammonia in soils are compared in Table II. A glance, first, at the Riverside soil will show that between 52 and 53 per cent of the nitrogen added was recovered by either method. In the Lordsburg soil the percentage of nitrogen recovered is much smaller than in the Riverside soil, and it would seem that the magnesium-oxid method was slightly more effective than the acid-extraction method, although the difference is small. It is observed that the ammonia fixed is practically the same whether the ammonia is added as a sulphate, chlorid, or nitrate. It would therefore appear that the anions have no effect on the fixation of ammonia.

TABLE II.—*Comparison of direct-distillation and acid-extraction methods for determining ammonia in soils*

Ammonium salts added.	Nitrogen added per 100 gm. of soil.	Nitrogen recovered.							
		Riverside soil.				Lordsburg soil.			
		Extracted with 10 per cent of hydrochloric acid.		Extracted with 10 gm. of magnesium oxid.		Extracted with 10 per cent of hydrochloric acid.		Extracted with 10 gm. of magnesium oxid.	
	Mgm.	*Mgm.*	*Per ct.*	*Mgm.*	*Per ct.*	*Mgm.*	*Per ct.*	*Mgm.*	*Per ct.*
Ammonium hydroxid	50.00	26.16	52.32	26.20	52.40	10.10	20.20	11.00	22.00
Ammonium nitrate	50.00	26.28	52.56	26.30	52.60	10.00	20.00	10.80	21.60
Ammonium chlorid	50.00	26.16	52.32	26.40	52.80	10.10	20.20	10.75	21.50
Ammonium sulphate	50.00	26.30	52.60	26.50	53.00	8.50	17.00	9.10	18.20

After it became evident that the ammonia could not be removed from the soil by extracting with acid or distilling with magnesium oxid, the soil was distilled with increasing amounts of potassium and sodium hydroxid. The results secured are presented in Table III. It is seen that the ammonia removed by boiling with potassium and sodium hydroxid is greater than the amount recovered by extracting with acid or distilling with magnesium oxid; but that much of the ammonia was retained by the soil, even when boiled with large quantities of potassium and sodium hydroxid. One c. c. of $10N$ potassium-hydroxid solution seemed to be less effective than 2.5 c. c., but the maximum removal of ammonia seems to have been secured when 2.5 c. c. of solution were added. In some cases the sodium hydroxid seems to have been more effective than the potassium solution; but this is possibly due to the fact that duplicate samples were quite variable, and, even though the results given are averages of several determinations, some allowance must be made for the lack of uniformity in duplicates. It is obvious that much of the ammonia fixed by this soil can not be removed by boiling with caustic substances, even when the compounds are added in great excess. When the soils were boiled with 10 per cent hydrochloric acid, more of the ammonia was removed than by extracting with cold hydrochloric acid of the same strength (Table IV). However, boiling for one hour left about one-third of the ammonia in the Riverside soil. When the boiling was continued for four hours, the ammonia removed varied from 86.4 to 88 per cent. The ammonia seems to have been more firmly fixed in the Lordsburg soil, as boiling for four hours with 10 per cent acid removed less than 75 per cent of the nitrogen added.

TABLE III.—*Recovery of ammonia from soils by distilling with potassium hydroxid and sodium hydroxid*

Quantity of potassium-hydroxid or sodium-hydroxid solution added.	Nitrogen in ammonium sulphate added per 100 gm. of soil.	Nitrogen recovered.			
		10N potassium hydroxid.		10N sodium hydroxid.	
C. c.	*Mgm.*	*Mgm.*	*Per cent.*	*Mgm.*	*Per cent.*
1	50.00	31.92	63.84	33.60	67.20
2	50.00	34.72	69.44	36.96	73.92
5	50.00	34.66	69.32	36.12	72.24
10	50.00	34.72	69.44	36.96	73.92
20	50.00	34.72	69.44	36.68	73.36
30	50.00	34.44	68.88	37.24	74.48
40	50.00	34.68	69.36	36.72	73.44
80	50.00	34.44	68.88	34.16	68.32
100	50.00	34.48	68.96	34.60	69.20

TABLE IV.—*Recovery of ammonia from soil by boiling with 10 per cent hydrochloric acid*

Time of boiling.	Nitrogen added per 100 gm. of soil.	Nitrogen recovered.											
		Riverside soil.						Lordsburg soil.					
		Ammonium sulphate.		Ammonium chlorid.		Ammonium nitrate.		Ammonium sulphate.		Ammonium chlorid.		Ammonium nitrate.	
Hours.	*Mgm.*	*Mgm.*	*P. ct.*	*Mgm.*	*P. ct.*	*Mgm.*	*P. ct.*	*Mgm.*	*P. ct.*	*Mgm.*	*P. ct.*	*Mgm.*	*P. ct.*
1	50.00	32.40	64.80	33.00	66.00	32.60	65.20	......	......	......	......	......	......
2	50.00	37.60	75.20	37.40	74.80	38.00	76.00	25.50	51.00	26.00	52.00	25.60	51.20
3	50.00	40.24	80.48	40.00	80.00	40.80	81.60	......	......	......	......	......	......
4	50.00	43.20	86.40	43.75	87.50	44.00	88.00	36.00	72.00	36.40	72.80	37.00	74.00

NITROGEN RECOVERED

The amount of ammonia recovered from soils by leaching with water or 10 per cent hydrochloric acid is shown in Table V.

TABLE V.—*Removal of ammonia from soils by leaching with water or 10 per cent hydrochloric acid*

Quantity of percolate.	Nitrogen in ammonium sulphate added per 100 gm. of soil.	Riverside soil.				Lordsburg soil.	
		Leached with water.		Leached with 10 per cent hydrochloric acid.		Leached with water.	
C. c.	*Mgm.*	*Mgm.*	*Per cent.*	*Mgm.*	*Per cent.*	*Mgm.*	*Per cent.*
400	50.00	11.42	22.84	25.80	51.60	4.40	8.80
800	50.00	14.63	29.26	34.04	68.08	5.30	10.60
1,200	50.00	15.54	31.08	37.68	75.36	5.90	11.80
1,600	50.00	16.15	32.30	39.06	78.12	6.50	13.00
2,000	50.00	16.39	32.78	40.06	80.12	6.90	13.80
2,400	50.00	16.50	33.00	40.60	81.20	7.10	14.20

The percolation of 400 c. c. of water through 100 gm. of soil removed 22.84 per cent of the ammonia from the Riverside soil and only 8.8 per cent from the Lordsburg soil. The percolation of the 400 c. c. of 10 per cent acid through the Riverside soil removed a little more than twice as much ammonia as the same amount of water. The leaching of the soils was continued until 2,400 c. c. of percolate had passed through. The total amount of ammonia removed by leaching with water amounted to only 14.2 per cent for the Lordsburg soil and 33 per cent for the Riverside soil.

The removal of ammonia by leaching with large quantities of 10 per cent acid was tried only with the Riverside soil, from which 2,400 c. c. removed 81.2 per cent of the ammonia added.

FACTORS INFLUENCING THE FIXATION OF AMMONIA BY SOILS

As shown in Table VI, the depth from which the soil sample is taken is frequently an important factor in the determination of the ammonia-fixing power of the soil. All of the semiarid soils tested, with the exception of the soil from Highland, Cal., show an increased fixation with an increase in depth. The humid soils do not show an increased fixation with depth, and it would therefore seem that there may be a marked difference between humid and semiarid soil in this regard. However, the number of soils examined are too few to warrant any conclusions at this time.

TABLE VI.—*Fixation of ammonia by soils from different depths*

Depth of soil.	Nitrogen in ammonium sulphate added per 100 gm. of soil.	Nitrogen recovered.													
		Lordsburg, Cal.		Riverside, Cal.		Covina, Cal.		Highland, Cal.		Berwyn, Md.		Arlington, Va.			
												Sample 1.		Sample 2.	
Inches.	*Mgm.*	*Mgm.*	*Per cent.*	*Mgm.*	*Per cent.*	*Mgm.*	*Per cent.*	*Mgm.*	*Per cent.*	*Mgm.*	*Per cent.*	*Mgm.*	*Per cent.*	*Mgm.*	*Per cent.*
0- 6 . . .	50.00	47.20	94.40	46.50	93.00	44.40	88.80	49.50	99.00	48.20	96.40	45.40	90.80	45.40	90.80
6-18 . . .	50.00	38.90	77.80	45.85	91.70	42.15	84.30	46.95	93.90	48.70	97.40	45.00	90.00	46.00	92.00
18-30 . . .	50.00	21.80	43.60	40.80	81.60	36.05	72.10	47.30	94.60	47.60	95.20	46.40	92.80	46.40	92.80
30-42 . . .	50.00	12.90	25.80	33.85	67.70	22.35	44.70	. . . . . .	. . . . . .	47.20	94.40	. . . . . .	. . . . . .	. . . . . .	. . . . . .
42-54 . . .	50.00	10.10	20.20	26.70	53.40	. . . . . .	. . . . . .	. . . . . .	. . . . . .	46.00	92.00	. . . . . .	. . . . . .	. . . . . .	. . . . . .

The addition of the same quantity of an ammonium salt in concentrated and dilute solution shows the greatest fixation when added in concentrated solution, as shown in Table VII. When dilute solutions were added, the containers were placed on a large wheel which completed about two revolutions per minute; thus, the soil was kept agitated dur-

ing the fixation period. However, it is observed that the nitrogen recovered varied from 52 to 53.5 per cent when 20 c. c. of solution were added and from 65 to 66 per cent when 720 c. c. of solution were added.

TABLE VII.—*Fixation of ammonia from concentrated and dilute solutions*

Quantity of solution added.	Nitrogen added per 100 gm. of soil.	Nitrogen recovered from ammonium sulphate.		Nitrogen recovered from ammonium chlorid.		Nitrogen recovered from ammonium nitrate.	
C. c.	*Mgm.*	*Mgm.*	*Per cent.*	*Mgm.*	*Per cent.*	*Mgm.*	*Per cent.*
20	50. 00	26. 10	52. 20	26. 25	53. 50	26. 00	52. 00
200	50. 00	27. 52	55. 04	28. 10	56. 20	27. 80	55. 60
360	50. 00	28. 25	56. 50	29. 00	58. 00	28. 80	57. 60
720	50. 00	32. 61	65. 22	32. 60	65. 20	33. 00	66. 00

If the fixation is dependent upon the chemical reactions, it would seem that the temperature would be an important factor in determining the amount of ammonia fixed. Table VIII shows the results secured with two soils when held at temperatures ranging from 5° to 100° C. In both soils the amount of ammonia recovered decreased as the temperature increased. In the Lordsburg soil the ammonia recovered when the soil was held at 100° is only 53.6 per cent of the amount recovered when the soil was held at 5°.

TABLE VIII.—*Effect of temperature on the fixation of ammonia by soils*

Temperature.	Nitrogen in ammonium sulphate added per 100 gm. of soil.	Nitrogen recovered.			
		Riverside soil.		Lordsburg soil.	
°C.	*Mgm.*	*Mgm.*	*Per cent.*	*Mgm.*	*Per cent.*
5	50. 00	27. 42	55. 44	12. 50	25. 00
20	50. 00	25. 48	50. 96	10. 10	20. 20
50	50. 00	20. 16	40. 32	9. 10	18. 20
75	50. 00	19. 32	38. 64	8. 80	17. 60
100	50. 00	17. 36	34. 72	6. 70	13. 40

The time of standing exerted some influence, as can be seen by referring to Table IX. As might be expected, the most rapid fixation takes place during the first few minutes after the ammonia is added; but the process is apparently not complete even after a period of 72 hours, as less ammonia was removed after 96 hours than after 72 hours; and in the Lordsburg soil the difference is quite appreciable.

TABLE IX.—*Effect of time on the fixation of ammonia by soils*

Time ammonia was allowed to remain in contact with the soil.	Nitrogen in ammonium sulphate added per 100 gm. of soil.	Nitrogen recovered.			
		Riverside soil.		Lordsburg soil.	
Hours.	*Mgm.*	*Mgm.*	*Per cent.*	*Mgm.*	*Per cent.*
½	50.00	20.82	41.64	10.10	20.20
1	50.00	26.50	53.00	9.90	19.80
4	50.00	25.46	50.92	9.10	18.20
8	50.00	24.02	48.04	7.90	15.80
16	50.00	23.00	46.00	7.70	15.40
24	50.00	22.57	45.14	7.75	15.50
48	50.00	21.84	43.68	7.80	15.60
72	50.00	21.26	42.52	7.60	15.20
96	50.00	20.97	41.94	6.60	13.20

Heating a soil to high temperatures caused marked changes in the chemical and physical nature of the soil, as shown in Table X. A temperature of 200° C. or less for six hours seems to have had very little effect, but 250° caused a marked reduction in the ammonia-fixing power of the soil, and a temperature of 300° reduced the fixation in the Riverside soil to 10 per cent and in the Lordsburg soil to 12.8 per cent.

TABLE X.—*Effect of heating the soil in a hot-air oven for six hours previous to the addition of ammonia*

Temperature.	Nitrogen added per 100 gm. of soil.	Nitrogen recovered.					
		Ammonium sulphate.				Ammonium chlorid.	
		Riverside soil.		Lordsburg soil.		Riverside soil.	
°C.	*Mgm.*	*Mgm.*	*Per cent.*	*Mgm.*	*Per cent.*	*Mgm.*	*Per cent.*
100	50.00	25.76	51.52	10.40	20.80	25.76	51.52
150	50.00	25.76	51.52	10.35	20.70	25.76	51.52
200	50.00	26.88	53.76	10.60	21.20	26.60	53.20
250	50.00	37.24	74.48	33.10	66.20	37.19	74.38
275	50.00	42.92	85.84	39.20	78.40	42.64	85.28
300	50.00	45.00	90.00	43.60	87.20	45.00	90.00

An examination of Table XI shows that the percentage of nitrogen as ammonia recovered remained constant until the amount of nitrogen added was greater than 25 mgm. per 100 gm. of Riverside soil. After this point the percentage fixation decreased, but the absolute fixation increased until about 23 mgm. per 100 gm. of soil were fixed, after which no further increase was secured. The percentage fixation in the Lordsburg soil remained constant until the nitrogen added amounted to more than 50 mgm. per 100 gm. of soil. When amounts of nitrogen greater

than 50 mgm. per 100 gm. of soil were added, the percentage fixation decreased as the amount added was increased. It would seem that the Riverside soil has the power of fixing about 900 pounds of nitrogen as ammonia per acre-foot, while the Lordsburg soil is capable of fixing more than 3,000 pounds. These figures are based upon the fixation which took place in 30 minutes. If the ammonia had been allowed to remain in contact with the soil for several days, the amount fixed would have been very greatly increased, as shown in Table IX.

TABLE XI.—*Effect of adding increasing amounts of ammonium sulphate on the fixation of ammonia by soils*

Nitrogen in ammonium sulphate added per 100 gm. of soil.	Riverside soil.			Lordsburg soil.		
	Nitrogen recovered.	Nitrogen fixed.	Nitrogen recovered.	Nitrogen recovered.	Nitrogen fixed.	Nitrogen recovered.
Mgm.	*Mgm.*	*Mgm.*	*Per cent.*	*Mgm.*	*Mgm.*	*Per cent.*
6.25	2.52	3.73	40.32	....	....	....
12.50	5.04	7.46	40.32	2.80	9.70	22.40
25.00	10.36	14.64	41.44	5.60	19.40	22.40
37.50	17.80	19.70	47.48	8.40	29.10	22.40
50.00	26.60	23.40	53.20	11.20	38.80	22.40
75.00	52.00	23.00	69.33	25.95	49.05	34.60
100.00	76.44	23.56	76.44	44.80	55.20	44.80
150.00	125.50	24.50	83.67	84.00	66.00	56.00
200.00	....	....	....	129.60	70.40	64.80
300.00	....	....	....	224.40	75.60	74.80
400.00	....	....	....	318.00	82.00	79.50

EFFECT OF ALUMINIUM, IRON, POTASSIUM, SODIUM, MAGNESIUM, AND CALCIUM SALTS ON THE FIXATION OF AMMONIA BY SOILS

The addition of aluminium salts to the soil 30 minutes prior to the addition of the ammonium salt caused a marked reduction in the ammonia-fixing power of the soil. Even the addition of 10 c. c. of *N/32* aluminium sulphate, chlorid, or nitrate reduced the ammonia-fixing power of the soil, as shown in Table XII. When 10 c. c. of *N/1* aluminium salts were added, about 90 per cent of the ammonia added was recovered. The addition of the aluminium salt 24 hours prior to the addition of the ammonium salts generally caused a greater reduction in fixation than when the aluminium salt was allowed to act but one-half hour. However, the time factor is of much less importance than the amount of salt added. The soluble iron and potassium salts, as shown in Tables XIII and XIV, also have a marked influence on the ammonia-fixing power of the soil tested. The potassium salts appear to have had somewhat less effect than the aluminium or iron salts when added in very small quantities; but, when added in larger quantities, these salts were quite as effective as the aluminium or iron salts.

TABLE XII.—*Effect of aluminium salts on the fixation of ammonia in a soil from Riverside, Cal.*

Time aluminium salt was allowed to stand before addition of ammonia.	Quantity and strength of aluminium salt added.	Nitrogen in ammonium sulphate added per 100 gm. of soil.	Nitrogen recovered.					
			Aluminium sulphate.		Aluminium chlorid.		Aluminium nitrate.	
Hours.		*Mgm.*	*Mgm.*	*Per cent.*	*Mgm.*	*Per cent.*	*Mgm.*	*Per cent.*
½	10 c. c. *N/1*	50.00	45.15	90.30	43.96	87.92	44.20	88.40
½	10 c. c. *N/2*	50.00	42.35	84.70	41.44	82.88	42.00	84.00
½	10 c. c. *N/4*	50.00	40.67	81.34	40.60	81.20	40.70	81.40
½	10 c. c. *N/8*	50.00	37.87	75.74	37.80	75.60	37.60	75.20
½	10 c. c. *N/16*	50.00	31.41	62.82	33.88	67.76	31.80	63.60
½	10 c. c. *N/32*	50.00	28.63	57.26	29.96	59.92	29.00	58.00
24	10 c. c. *N/1*	50.00	47.00	94.00	46.70	93.40	46.80	93.60
24	10 c. c. *N/2*	50.00	44.52	89.04	44.60	89.20	44.80	89.60
24	10 c. c. *N/4*	50.00	42.56	85.12	42.70	85.40	42.60	85.20
24	10 c. c. *N/8*	50.00	38.92	77.84	38.90	77.80	39.00	78.00
24	10 c. c. *N/16*	50.00	33.60	67.20	33.40	66.80	33.60	67.20
24	10 c. c. *N/32*	50.00	28.84	57.68	28.60	57.20	28.70	57.40

TABLE XIII.—*Effect of adding iron salts on the fixation of ammonia in a soil from Riverside, Cal.*

Time iron salt was allowed to stand before addition of ammonia.	Quantity and strength of iron salt added.	Nitrogen in ammonium sulphate added per 100 gm. of soil.	Nitrogen recovered.			
			Iron chlorid.		Iron nitrate.	
Hours.		*Mgm.*	*Mgm.*	*Per cent.*	*Mgm.*	*Per cent.*
½	10 c. c. *N/1*	50.00	43.96	87.92	45.71	91.42
½	10 c. c. *N/2*	50.00	42.28	84.56	43.75	87.50
½	10 c. c. *N/4*	50.00	41.16	82.32	40.39	80.78
½	10 c. c. *N/8*	50.00	36.12	72.24	34.23	68.46
½	10 c. c. *N/16*	50.00	32.20	64.40	24.33	48.66
½	10 c. c. *N/32*	50.00	29.40	58.80	27.80	55.60
24	10 c. c. *N/1*	50.00	47.32	94.64	47.00	94.00
24	10 c. c. *N/2*	50.00	46.20	92.40	46.30	92.60
24	10 c. c. *N/4*	50.00	41.32	82.64	40.90	81.80
24	10 c. c. *N/8*	50.00	34.44	68.88	34.80	69.60
24	10 c. c. *N/16*	50.00	31.20	62.40	31.40	62.80
24	10 c. c. *N/32*	50.00	28.70	57.40	28.60	57.20

TABLE XIV.—*Effect of adding potassium salts on the fixation of ammonia in soils from Riverside, Cal.*

Time potassium salt was allowed to act before the addition of ammonia.	Quantity and strength of potassium salt added.	Nitrogen in ammonium sulphate added per 100 gm. of soil.	Nitrogen recovered.					
			Potassium sulphate.		Potassium chlorid.		Potassium nitrate.	
Hours.		*Mgm.*	*Mgm.*	*Per cent.*	*Mgm.*	*Per cent.*	*Mgm.*	*Per cent.*
½	10 c. c. *N/1*	50.00	47.09	94.18	47.48	94.96	47.09	94.18
½	10 c. c. *N/2*	50.00	42.56	85.12	42.56	85.12	42.74	85.48
½	10 c. c. *N/4*	50.00	34.72	69.44	34.72	69.44	34.72	69.44
½	10 c. c. *N/8*	50.00	30.80	61.60	30.80	61.60	30.80	61.60
½	10 c. c. *N/16*	50.00	28.00	56.00	28.00	56.00	28.00	56.00
½	10 c. c. *N/32*	50.00	26.60	53.20	26.60	53.20	26.60	53.20
24	10 c. c. *N/1*	50.00	47.32	94.64	47.25	94.50	47.40	94.80
24	10 c. c. *N/2*	50.00	42.56	85.12	43.00	86.00	42.60	85.20
24	10 c. c. *N/4*	50.00	38.64	77.28	38.60	77.20	38.50	77.00
24	10 c. c. *N/8*	50.00	32.48	64.96	32.50	65.00	32.60	65.20
24	10 c. c. *N/16*	50.00	29.12	58.24	29.10	58.20	29.10	58.20
24	10 c. c. *N/32*	50.00	27.72	55.44	27.60	55.20	27.80	55.60

The action of sodium salts on the fixation of ammonia is quite different from the action of potassium salts, as can be readily seen by comparing Tables XIV and XV. The addition of sodium salts seems to have slightly reduced the ammonia-fixing power of the soil. When the sodium salts were added 24 hours prior to the addition of the ammonia, the reduction in the fixing power of the soil was no greater than when the sodium salt was allowed to act only one-half hour. The addition of magnesium and calcium salts, like the sodium salts, caused very little change in the ammonia-fixing power of the soil, even when the salts were added in considerable amounts (Tables XVI and XVII).

It appears that the anions have little or no effect in determining the action of a salt on the ammonia-fixing power of a soil. The three cations which had a marked influence on the ammonia-fixing power of a soil were equally effective, regardless of the anion with which they were combined; and the action of the three cations which had little effect was apparently not influenced by the anions with which they were combined.

TABLE XV.—*Effect of adding sodium salts on the fixation of ammonia by a soil from Riverside, Cal.*

Time sodium salt was allowed to stand before the addition of ammonia.	Quantity and strength of sodium salt added.	Nitrogen in ammonium sulphate added per 100 gm. of soil.	Nitrogen recovered.					
			Sodium sulphate.		Sodium chlorid.		Sodium nitrate.	
Hours.		*Mgm.*	*Mgm.*	*Per cent.*	*Mgm.*	*Per cent.*	*Mgm.*	*Per cent.*
½	10 c. c. *N/1*	50.00	27.24	54.48	27.16	54.32	27.46	54.92
½	10 c. c. *N/2*	50.00	27.10	54.20	27.20	54.40	27.26	54.52
½	10 c. c. *N/4*	50.00	27.10	54.20	27.16	54.32	27.16	54.32
½	10 c. c. *N/8*	50.00	27.30	54.60	27.24	54.48	27.06	54.12
½	10 c. c. *N/16*	50.00	27.15	54.30	27.36	54.72	27.20	54.40
½	10 c. c. *N/32*	50.00	27.20	54.40	27.16	54.32	27.16	54.32
24	10 c. c. *N/1*	50.00	26.80	53.60	26.76	53.52	26.10	52.20
24	10 c. c. *N/2*	50.00	26.00	52.00	26.76	53.52	26.00	52.00
24	10 c. c. *N/4*	50.00	26.75	53.50	26.76	53.52	26.80	53.60
24	10 c. c. *N/8*	50.00	26.90	53.80	26.48	52.96	26.90	53.80
24	10 c. c. *N/16*	50.00	26.00	52.00	26.34	52.68	26.75	53.50
24	10 c. c. *N/32*	50.00	26.85	53.70	26.20	52.40	26.90	53.80

TABLE XVI.—*Effect of adding magnesium salts on the fixation of ammonia in a soil from Riverside, Cal.*

Time magnesium salt was allowed to stand before the addition of ammonia.	Quantity and strength of magnesium salt added.	Nitrogen in ammonium sulphate added per 100 gm. of soil.	Nitrogen recovered.					
			Magnesium sulphate.		Magnesium chlorid.		Magnesium nitrate.	
Hour.		*Mgm.*	*Mgm.*	*Per cent.*	*Mgm.*	*Per cent.*	*Mgm.*	*Per cent.*
½	10 c. c. *N/2*	50.00	26.88	53.76	26.74	53.48	26.88	53.76
½	10 c. c. *N/4*	50.00	26.70	53.40	26.70	53.40	27.00	54.00
½	10 c. c. *N/8*	50.00	26.60	53.20	26.60	53.20	26.70	53.40
½	10 c. c. *N/16*	50.00	26.40	52.80	26.80	53.60	26.60	53.20
½	10 c. c. *N/32*	50.00	26.32	52.64	26.60	53.20	26.80	53.60

TABLE XVII.—*Effect of adding calcium salts on the fixation of ammonia by a soil from Riverside, Cal.*

Time calcium salt was allowed to stand before the addition of ammonia.	Quantity and strength of calcium salt added.	Nitrogen in ammonium sulphate added per 100 gm. of soil.	Nitrogen recovered.					
			Calcium sulphate.		Calcium chlorid.		Calcium nitrate.	
Hour.		*Mgm.*	*Mgm.*	*Per cent.*	*Mgm.*	*Per cent.*	*Mgm.*	*Per cent.*
½	10 c. c. *N/1*	50.00	26.70	53.40	26.60	53.20	26.80	53.60
½	10 c. c. *N/2*	50.00	26.80	53.60	26.32	52.64	27.16	54.32
½	10 c. c. *N/4*	50.00	26.80	53.60	26.40	53.80	26.70	53.50
½	10 c. c. *N/8*	50.00	26.50	53.00	26.70	53.40	26.60	53.20
½	10 c. c. *N/16*	50.00	26.40	52.80	26.50	53.00	26.40	52.80
½	10 c. c. *N/32*	50.00	26.60	53.20	26.40	52.80	26.30	52.60

Table XVIII shows that the three salts which had little influence on the fixation of ammonia in the soil from Riverside, Cal., also had little effect on the ammonia-fixing power of the soil from Lordsburg, Cal., while the iron, potassium, and aluminium salts, which had a marked influence on the ammonia-fixing power of the Riverside soil, were also effective in the Lordsburg soil. It would therefore seem that the process of fixation in these two soils is somewhat closely related. However, the relative effectiveness of the iron, potassium, and aluminium salts appears to be somewhat different in the two soils.

TABLE XVIII.—*Effect of nitrate salts on the fixation of ammonia by a Lordsburg soil*

Time nitrate salts were allowed to stand before the addition of ammonia.	Quantity and strength of nitrate salt added.	Nitrogen in ammonium sulphate added per 100 gm. of soil.	Nitrogen recovered.											
			Calcium nitrate.		Magnesium nitrate.		Sodium nitrate.		Ferric nitrate.		Potassium nitrate.		Aluminium nitrate.	
Hour.		*Mgm.*	*Mgm.*	*P. ct.*	*Mgm.*	*P. ct.*	*Mgm.*	*P. ct.*	*Mgm.*	*P. ct.*	*Mgm.*	*P. ct.*	*Mgm.*	*P. ct.*
½	5 c. c. *N*/2	50.00	11.60	23.20	11.50	23.00	11.40	22.80	33.90	67.80	22.00	44.00	38.00	76.00
½	10 c. c. *N*/2	50.00	11.80	23.60	11.70	23.40	11.40	22.80	42.00	84.00	33.50	67.00	41.60	83.20

EFFECT OF SALTS ON THE SOLUBILITY OF CALCIUM IN SOILS

During the course of the work on the fixation of ammonia by soils it was observed that large quantities of calcium were brought into solution, and it was thought that the calcium dissolved by the addition of ammonium salts might bear some relation to the ammonia fixed by the soil. Table XIX shows the effect of ammonium, aluminium, sodium, and magnesium chlorids on the solubility of calcium in semiarid and humid soils. It is seen that the solutions of these salts invariably dissolved a much larger quantity of calcium than did distilled water.

TABLE XIX.—*Effect of ammonium, aluminium, sodium, and magnesium chlorid on the solubility of calcium in semiarid and humid soils*

[Results stated as milligrams of calcium per 100 gm. of soil]

Locality of sample.	Depth from which sample was taken.	Quantity of each salt solution added.	Water only.	Increase over water only.						
				NH_4Cl.	$AlCl_3$.	$AlCl_3 + NH_4Cl$.	NaCl.	$NaCl + NH_4Cl$.	$MgCl_2$.	$MgCl_2 + NH_4Cl$.
	Inches.									
Lordsburg, Cal.	0–6	7.5 c. c. *N*/2 of each salt solution per 100 gm. of soil.	6.12	18.90	126.18	130.08	9.30	24.18	30.18	39.48
Do.	6–18	do.	4.2	27.72	119.70	123.60	10.50	30.00	31.02	47.22
Do.	18–30	do.	4.5	32.22	116.82	126.42	10.62	36.00	27.60	54.60
Do.	30–42	do.	4.35	37.95	120.30	159.75	11.10	43.50	29.70	60.45
Riverside, Cal.	0–6	do.	6.45	29.85	113.70	114.75	8.55	22.65	30.90	39.75
Do.	54–66	do.	4.05	36.30	110.55	112.50	7.95	41.55	29.40	56.55
Berwyn, Md.	0–6	do.	7.50	13.50	43.20					
Do.	6–18	do.	4.80	6.30	42.00					
Do.	18–30	do.	3.00	6.00	18.30					

Of the five salts used, aluminium chlorid gave the maximum effect, while the minimum effect was found with the sodium chlorid. The addition of an ammonium salt to a nonammoniacal salt gave a greater effect than when either of the salts was used alone. It is also observed that the increase in the solubility of calcium due to the addition of aluminium, sodium, or magnesium chlorid is fairly constant for the different depths, while the amount of calcium dissolved by the ammonium salts when used alone or in combination with other salts increases with the depth in the semiarid but not in the humid soils. This would seem to indicate a possible relation between the removal of calcium and the fixation of ammonia; but the data collected are too limited to warrant any definite conclusion at this time.

SUMMARY

(1) Many semiarid subsoils have the property of fixing large quantities of ammonia. Much of the ammonia fixed can not be removed by the ordinary methods for determining the ammonia content of the soils.

(2) Extracting the soil with 10 per cent hydrochloric acid gives approximately the same quantity of ammonia as distilling the soils with magnesium oxid.

(3) Anions apparently have little or no influence on the fixation of ammonia by soils.

(4) The ammoniacal nitrogen removed from duplicate samples of soil extracted with 10 per cent acid gives remarkably consistent results, while duplicate samples of soil distilled at atmospheric pressure with magnesium oxid frequently fail to give a satisfactory agreement.

(5) A large percentage of the ammonia added to semiarid soils and subsoils can not be recovered by boiling the soil with excessive amounts of caustic solutions.

(6) Boiling soils with 10 per cent hydrochloric acid removes practically all of the ammoniacal nitrogen from one soil studied, but less than 75 per cent was recovered from another soil.

(7) The fixation of ammonia by semiarid soils increases with depth. In this regard semiarid soils appear to differ from humid soils.

(8) The addition of ammonium salts in a concentrated solution results in greater fixation than when the same amount is added in a dilute solution.

(9) The fixation of ammonia by soils increases with the temperature.

(10) The fixation of ammonia by a soil is most rapid during the first few minutes, but the fixation process appears to continue for several days.

(11) Heating a soil for six hours at temperatures of 200° C. or above reduces its power of fixing ammonia.

(12) When small amounts of ammonium salts are added, the percentage of ammonia fixed remains constant. If increasing amounts of ammonia are added, a point is reached at which the percentage fixation becomes less, but the absolute fixation may continue to increase.

(13) Aluminium, iron, and potassium salts added to soils prior to the addition of ammonia reduce the ammonia-fixing power of the soils very decidedly.

Calcium, magnesium, and sodium salts added to the semiarid soils prior to the addition of the ammonia have little effect on the ammonia-fixing power of semiarid soils.

(14) The anions of aluminium, iron, potassium, calcium, sodium, or magnesium salts apparently have no influence on the action of these salts in reducing the ammonia-fixing power of semiarid soils.

(15) In semiarid soils the quantity of calcium brought into solution by ammonium chlorid increases with depth; when extracted with aluminium, sodium, or magnesium chlorid, the calcium brought into solution does not increase with the depth.

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